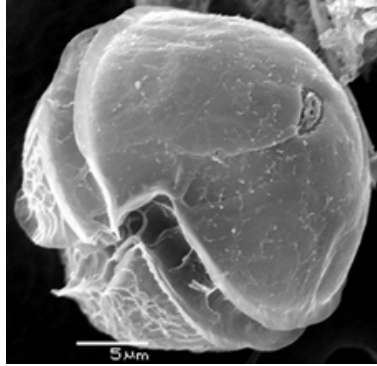

**CRISPR-cas9 Gene Drive system to remove
Saxitoxin production pathway from Algal Blooms
of *Alexandrium fundyense***

Algal Bloom Doom

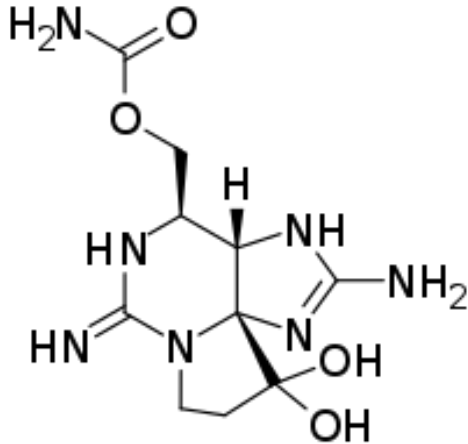
Andrew Song



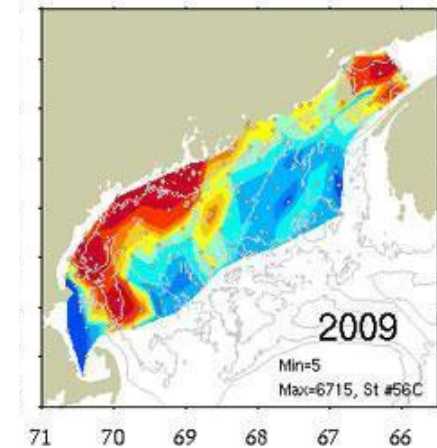
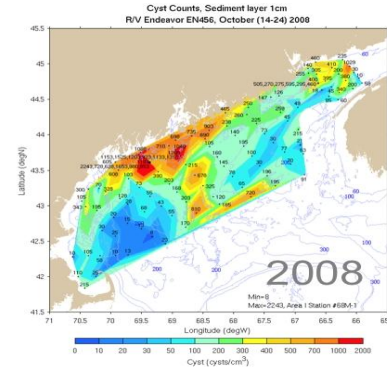
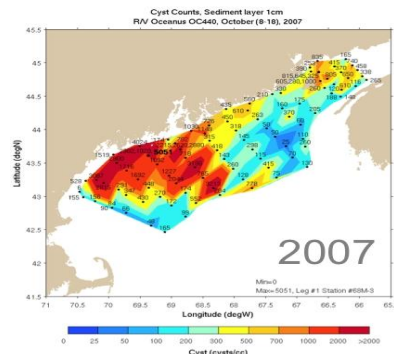
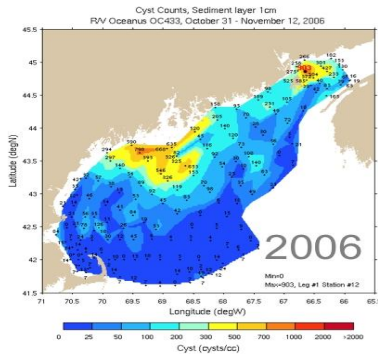
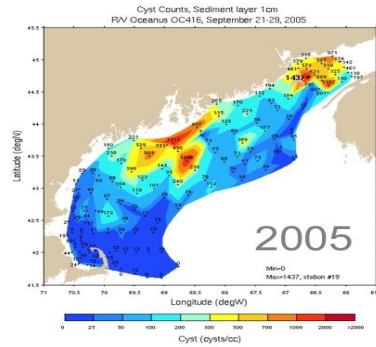
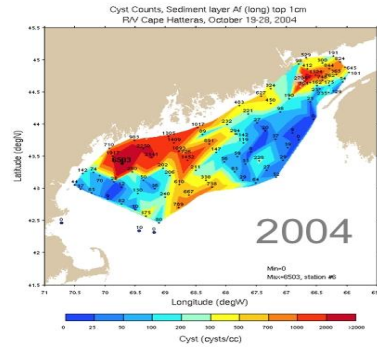
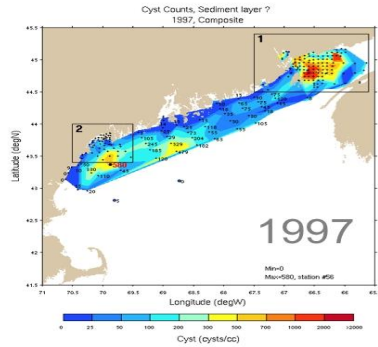


The Problem:

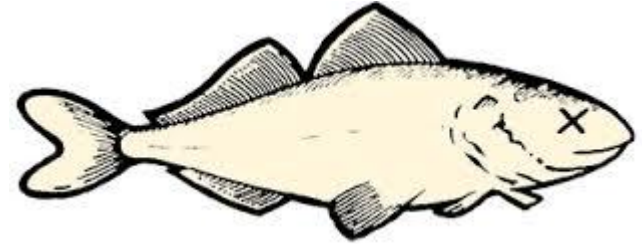
- HAB (Harmful algal blooms), specifically those caused by *Alexandrium fundyense*
- *A. fundyense* releases saxitoxin, a potent neurotoxin that causes PSP (Paralytic Shellfish Poisoning)
- Fishing zones with high levels of saxitoxin are banned; *A. fundyense* blooms close off shellfishing waters worth an annual \$50 million in the Gulf of Maine
- (Total loss from all algal blooms in the US amount to \$2.2-4.6 billion)



Algal blooms are on the Rise

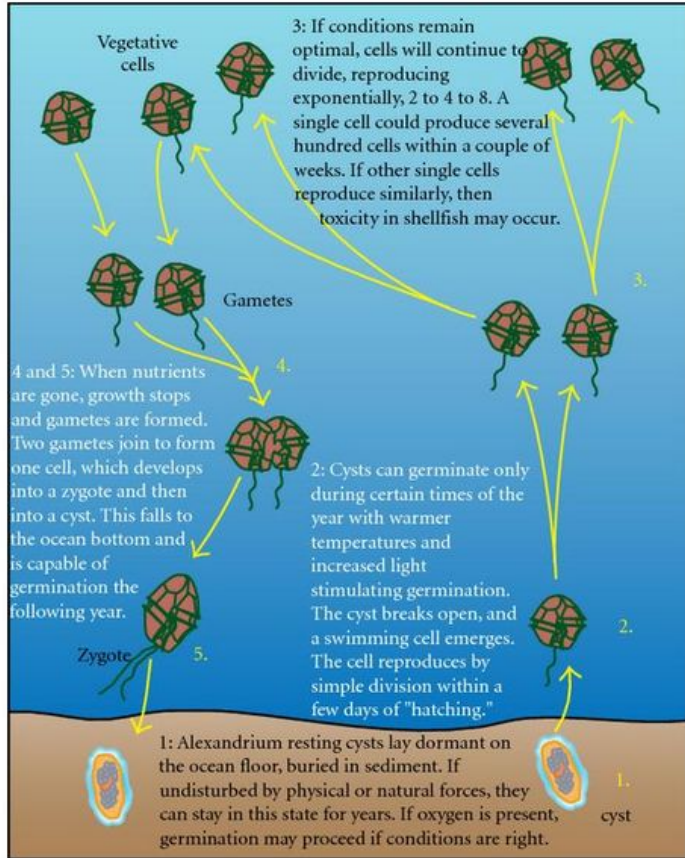


Solutions: None



- Algaecides are toxic to fish
- Monitoring devices such as the Environmental Sample Processor can detect algae
- Solid-phase adsorption toxin tracking (SPATT) can detect algal toxin concentrations





A brief guide to *Alexandrium*

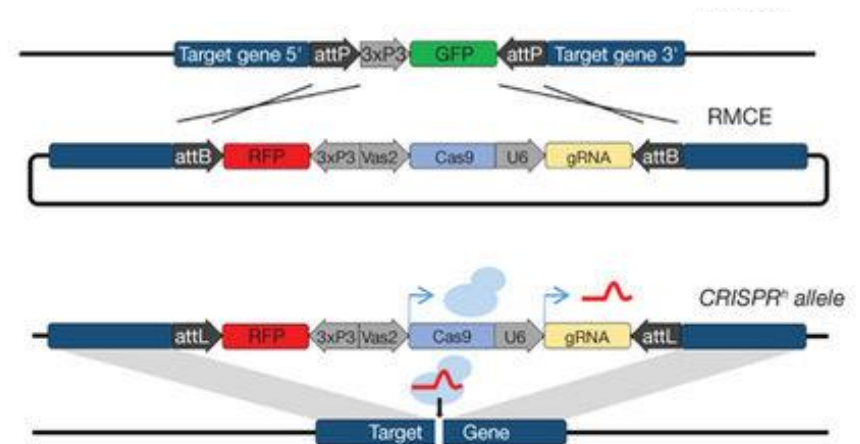
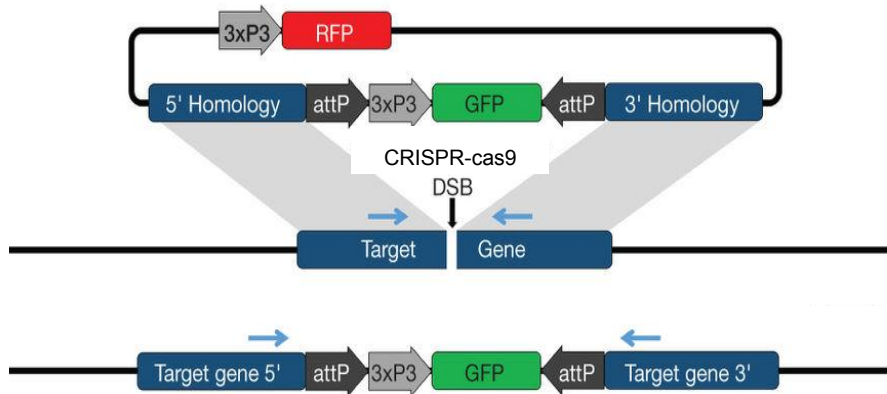
- Photosynthetic marine dinoflagellate
- Inhabits shallow waters
- Produces both asexually and sexually
- Optimal temperature 13-17 C



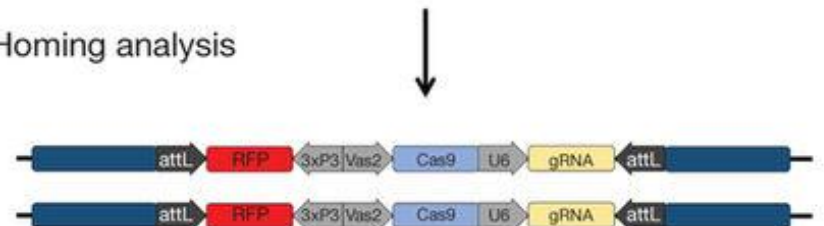
Solution: CRISPR/cas9 Gene Drive system

- Knock out JF343238.1, gene responsible for part of the saxitoxin synthesis pathway in *A. fundyense* using CRISPR/cas9
 - Insert cas9 and JF343238.1-specific gRNA coding sequences by using RMCE (Recombinase-mediated cell exchange)
-

Design



Homing analysis

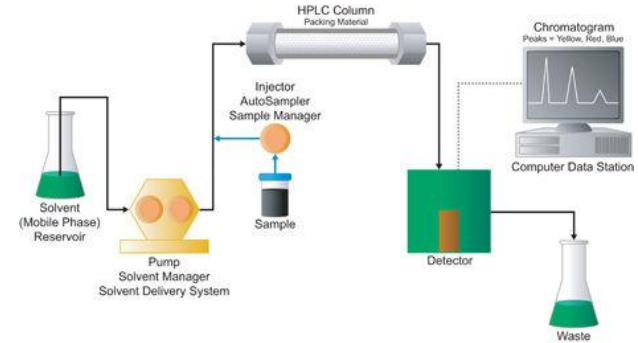


Expected Results...

Modified <i>A. fundyense</i>	Wild-type <i>A. fundyense</i>	Saxitoxin
0	0	0
0	1	1
1	0	0
1	1	1

(Asexual reproduction only)

- Without sexual reproduction, saxitoxin is present when WT is present
- If modified variant is added to a colony of WT and conditions are harsh, sexual reproduction will begin
- After multiple generations (depending on initial ratio of modified to WT), saxitoxin will not be detected



(High pressure liquid chromatography)

Advantages

- Rather cheap, since one successful modified cell line can be used to produce millions cells
 - Actually removes saxitoxins (albeit over time) rather than simply avoiding them
 - Gives back access to major fishing zones
 - Can be used with pre-existing technology
-

Possible Problems



- CRISPR-cas9 recognizes a specific sequence; this sequence can appear in another part of the genome other than the desired locus
 - JF343238.1 could code for proteins with other functions
 - Evolution
-

Any Questions?



Sources

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